

DSP-12 AND DSP-13 DUAL-SPECIFICITY PHOSPHATASES

CROSS-REFERENCE TO RELATED APPLICATION

5 This application claims the benefit of USSN 60 179,886, filed February 2, 2000, which is incorporated herein by reference in its entirety.

10 The present invention relates generally to compositions and methods useful for treating conditions associated with defects in cell proliferation, cell differentiation and/or cell survival. The invention is more particularly related to dual-specificity protein phosphatases, and polypeptide variants thereof. The present invention is also related to the use of such polypeptides to identify antibodies and other agents, including small molecules, that modulate signal transduction leading to proliferative responses, cell differentiation and/or cell survival.

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Background of the Invention

 Mitogen-activated protein kinases (MAP-kinases) are present as components of conserved cellular signal transduction pathways that have a variety of conserved members. MAP-kinases are activated by phosphorylation at a dual
20 phosphorylation motif with the sequence Thr-X-Tyr (by MAP-kinase kinases), in which phosphorylation at the tyrosine and threonine residues is required for activity. Activated MAP-kinases phosphorylate several transduction targets, including transcription factors. Inactivation of MAP-kinases is mediated by dephosphorylation at this site by dual-specificity phosphatases referred to as MAP-kinase phosphatases. In
25 higher eukaryotes, the physiological role of MAP-kinase signaling has been correlated with cellular events such as proliferation, oncogenesis, development and differentiation. Accordingly, the ability to regulate signal transduction via these pathways could lead to the development of treatments and preventive therapies for human diseases associated

Dual-specificity protein tyrosine phosphatases (dual-specificity phosphatases) are phosphatases that dephosphorylate both phosphotyrosine and phosphothreonine/serine residues (Walton et al., *Ann. Rev. Biochem.* 62:101-120, 1993). Several dual-specificity phosphatases that inactivate a MAP-kinase have been identified, including MKP-1 (WO 97 00315; Keyse and Emslie, *Nature* 359:644-647, 1992), MKP-4, MKP-5, MKP-7, Hb5 (WO 97 06245), PACT (Ward et al., *Nature* 367:651-654, 1994), HVH2 (Guan and Butch, *J. Biol. Chem.* 270:7197-7203, 1995), PYSF1 (Groom et al., *EMBO J.* 15:3621-3632, 1996) and others (see, e.g., WO 95 21923). Expression of certain dual-specificity phosphatases is induced by stress or mitogens, but others appear to be expressed constitutively in specific cell types. The regulation of dual-specificity phosphatase expression and activity is critical for control of MAP-kinase mediated cellular functions, including cell proliferation, cell differentiation and cell survival. For example, dual-specificity phosphatases may function as negative regulators of cell proliferation. It is likely that there are many such dual-specificity phosphatases, with varying specificity with regard to cell type or activation. However, the regulation of dual specificity phosphatases remains poorly understood and only a relatively small number of dual-specificity phosphatases have been identified.

Accordingly, there is a need in the art for an improved understanding of MAP-kinase signaling, and the regulation of dual-specificity phosphatases within MAP-kinase signaling cascades. An increased understanding of dual-specificity phosphatase regulation may facilitate the development of methods for modulating the activity of proteins involved in MAP-kinase cascades, and for treating conditions associated with such cascades. The present invention fulfills these needs and further provides other related advantages.

Summary of the Invention

Briefly stated, the present invention provides compositions and methods

of DSP-12 recited in SEQ ID NO:2, or a variant thereof that differs in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of the residues in SEQ ID NO:2, such that the polypeptide retains the ability to dephosphorylate an activated MAP-kinase.

5 Within further aspects, the present invention provides an isolated polynucleotide that encodes at least ten consecutive amino acids of a polypeptide having a sequence corresponding to SEQ ID NO:2. In certain embodiments the invention provides an isolated polynucleotide that encodes at least fifteen consecutive amino acids of a polypeptide having a sequence corresponding to SEQ ID NO:2.
10 Certain such polynucleotides encode a DSP-12 polypeptide. Still further, polynucleotides may be antisense polynucleotides that comprise at least 15 consecutive nucleotides complementary to a portion of a DSP-12 polynucleotide and/or that detectably hybridize to the complement of the sequence recited in SEQ ID NO:1 under conditions that include a wash in 0.1X SSC and 0.1% SDS at 60 C for 15 minutes.
15 Also provided are expression vectors comprising any of the foregoing polynucleotides, and host cells transformed or transfected with such expression vectors.

 The present invention further provides, within other aspects, methods for producing a DSP-12 polypeptide, comprising the steps of: (a) culturing a host cell as described above under conditions that permit expression of the DSP-12 polypeptide;
20 and (b) isolating DSP-12 polypeptide from the host cell culture.

 Also provided by the present invention are isolated antibodies, and antigen binding fragments thereof, that specifically bind to a DSP-12 polypeptide such as a polypeptide having the sequence of SEQ ID NO:2.

 The present invention further provides, within other aspects,
25 pharmaceutical compositions comprising a polypeptide, polynucleotide, antibody or fragment thereof as described above in combination with a physiologically acceptable carrier.

 Within further aspects, the present invention provides methods for

and for a time sufficient to allow formation of an antibody DSP-12 complex; and (b) detecting the level of antibody DSP-12 complex.

Within still other aspects, the present invention provides methods for detecting DSP-12 expression in a sample, comprising: (a) contacting a sample with an antisense polynucleotide as described above; and (b) detecting in the sample an amount of DSP-12 polynucleotide that hybridizes to the antisense polynucleotide. The amount of DSP-12 polynucleotide that hybridizes to the antisense polynucleotide may be determined, for example, using polymerase chain reaction or a hybridization assay.

The invention also provides DSP-12 polypeptides useful in screening assays for modulators of enzyme activity and or substrate binding. Methods are also provided, within other aspects, for screening for an agent that modulates DSP-12 activity, comprising the steps of: (a) contacting a candidate agent with a polypeptide as described above, under conditions and for a time sufficient to permit interaction between the polypeptide and candidate agent; and (b) subsequently evaluating the ability of the polypeptide to dephosphorylate a DSP-12 substrate, relative to a predetermined ability of the polypeptide to dephosphorylate the DSP-12 substrate in the absence of candidate agent. Such methods may be performed *in vitro* or in a cellular environment (e.g., within an intact cell).

Within further aspects, methods are provided for screening for an agent that modulates DSP-12 activity, comprising the steps of: (a) contacting a candidate agent with a cell comprising a DSP-12 promoter operably linked to a polynucleotide encoding a detectable transcript or protein, under conditions and for a time sufficient to permit interaction between the promoter and candidate agent; and (b) subsequently evaluating the expression of the polynucleotide, relative to a predetermined level of expression in the absence of candidate agent.

Also provided are methods for modulating a proliferative response in a cell, comprising contacting a cell with an agent that modulates DSP-12 activity.

Within further aspects, the present invention provides methods for modulating DSP-12 activity in a cell, comprising contacting a cell with an agent that modulates DSP-12 activity.

The present invention further provides methods for modulating cell survival, comprising contacting a cell with an agent that modulates DSP-12 activity.

Within related aspects, the present invention provides methods for treating a patient afflicted with a disorder associated with DSP-12 activity (or treatable by administration of DSP-12), comprising administering to a patient a therapeutically effective amount of an agent that modulates DSP-12 activity. Such disorders include Duchenne muscular dystrophy, cancer, graft-versus-host disease, autoimmune diseases, allergies, metabolic diseases, abnormal cell growth, abnormal cell proliferation and cell cycle abnormalities.

Within further aspects, DSP-12 substrate trapping mutant polypeptides are provided. Such polypeptides differ from the sequence recited in SEQ ID NO:2 in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of the residues in SEQ ID NO:2, such that the polypeptide binds to a substrate with an affinity that is not substantially diminished relative to DSP-12, and such that the ability of the polypeptide to dephosphorylate a substrate is reduced relative to DSP-12. Within certain specific embodiments, a substrate trapping mutant polypeptide contains a substitution at position 222 or position 253 of SEQ ID NO:2.

The present invention further provides, within other aspects, methods for screening a molecule for the ability to interact with DSP-12, comprising the steps of: (a) contacting a candidate molecule with a polypeptide as described above under conditions and for a time sufficient to permit the candidate molecule and polypeptide to interact; and (b) detecting the presence or absence of binding of the candidate molecule to the polypeptide. The step of detecting may comprise, for example, an affinity purification step, a yeast two hybrid screen or a screen of a phage display library.

In another aspect, the present invention provides isolated DSP-13 polypeptides having the sequence of DSP-13 recited in SEQ ID NO:6, or a variant thereof that differs in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of the residues in SEQ ID NO:6, such that the

Within further aspects, the present invention provides an isolated polynucleotide that encodes at least ten consecutive amino acids of a polypeptide having a sequence corresponding to SEQ ID NO:6. In certain embodiments the invention provides an isolated polynucleotide that encodes at least fifteen consecutive amino acids of a polypeptide having a sequence corresponding to SEQ ID NO:6. Certain such polynucleotides encode a DSP-13 polypeptide. Still further, polynucleotides may be antisense polynucleotides that comprise at least 15 consecutive nucleotides complementary to a portion of a DSP-13 polynucleotide and/or that detectably hybridize to the complement of the sequence recited in SEQ ID NO:5 under conditions that include a wash in 0.1X SSC and 0.1% SDS at 60 °C for 15 minutes. Also provided are expression vectors comprising any of the foregoing polynucleotides, and host cells transformed or transfected with such expression vectors.

The present invention further provides, within other aspects, methods for producing a DSP-13 polypeptide, comprising the steps of: (a) culturing a host cell as described above under conditions that permit expression of the DSP-13 polypeptide; and (b) isolating DSP-13 polypeptide from the host cell culture.

Also provided by the present invention are isolated antibodies, and antigen binding fragments thereof, that specifically bind to a DSP-13 polypeptide such as a polypeptide having the sequence of SEQ ID NO:6.

The present invention further provides, within other aspects, pharmaceutical compositions comprising a polypeptide, polynucleotide, antibody or fragment thereof as described above in combination with a physiologically acceptable carrier.

Within further aspects, the present invention provides methods for detecting DSP-13 expression in a sample, comprising: (a) contacting a sample with an antibody or an antigen-binding fragment thereof as described above, under conditions and for a time sufficient to allow formation of an antibody-DSP-13 complex; and (b) detecting the level of antibody-DSP-13 complex.

antisense polynucleotide as described above; and (b) detecting in the sample an amount of DSP-13 polynucleotide that hybridizes to the antisense polynucleotide. The amount of DSP-13 polynucleotide that hybridizes to the antisense polynucleotide may be determined, for example, using polymerase chain reaction or a hybridization assay.

5 The invention also provides DSP-13 polypeptides useful in screening assays for modulators of enzyme activity and or substrate binding. Methods are also provided, within other aspects, for screening for an agent that modulates DSP-13 activity, comprising the steps of: (a) contacting a candidate agent with a polypeptide as described above, under conditions and for a time sufficient to permit interaction
10 between the polypeptide and candidate agent; and (b) subsequently evaluating the ability of the polypeptide to dephosphorylate a DSP-13 substrate, relative to a predetermined ability of the polypeptide to dephosphorylate the DSP-13 substrate in the absence of candidate agent. Such methods may be performed *in vitro* or in a cellular environment (e.g., within an intact cell).

15 Within further aspects, methods are provided for screening for an agent that modulates DSP-13 activity, comprising the steps of: (a) contacting a candidate agent with a cell comprising a DSP-13 promoter operably linked to a polynucleotide encoding a detectable transcript or protein, under conditions and for a time sufficient to permit interaction between the promoter and candidate agent; and (b) subsequently
20 evaluating the expression of the polynucleotide, relative to a predetermined level of expression in the absence of candidate agent.

Also provided are methods for modulating a proliferative response in a cell, comprising contacting a cell with an agent that modulates DSP-13 activity.

25 Within further aspects, methods are provided for modulating differentiation of a cell, comprising contacting a cell with an agent that modulates DSP-13 activity.

The present invention further provides methods for modulating cell growth, comprising contacting a cell with an agent that modulates DSP-13 activity.

by administration of DSP-13), comprising administering to a patient a therapeutically effective amount of an agent that modulates DSP-13 activity. Such disorders include Duchenne muscular dystrophy, cancer, graft-versus-host disease, autoimmune diseases, allergies, metabolic diseases, abnormal cell growth, abnormal cell proliferation and cell cycle abnormalities.

Within further aspects, DSP-13 substrate trapping mutant polypeptides are provided. Such polypeptides differ from the sequence recited in SEQ ID NO:6 in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of the residues in SEQ ID NO:6, such that the polypeptide binds to a substrate with an affinity that is not substantially diminished relative to DSP-13, and such that the ability of the polypeptide to dephosphorylate a substrate is reduced relative to DSP-13. Within certain specific embodiments, a substrate trapping mutant polypeptide contains a substitution at position 368 or position 399 of SEQ ID NO:6.

The present invention further provides, within other aspects, methods for screening a molecule for the ability to interact with DSP-13, comprising the steps of: (a) contacting a candidate molecule with a polypeptide as described above under conditions and for a time sufficient to permit the candidate molecule and polypeptide to interact; and (b) detecting the presence or absence of binding of the candidate molecule to the polypeptide. The step of detecting may comprise, for example, an affinity purification step, a yeast two hybrid screen or a screen of a phage display library.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

Brief Description of the Drawings

Figure 1 illustrates a DNA sequence encoding DSP-13 (SEQ ID NO:1).

Figure 2 presents the predicted amino acid sequence of DSP-12 (SEQ ID NO:2).

Figure 3 presents a cDNA sequence for DSP-13 (SEQ ID NO:5), with the start and stop codons indicated in bold.

5 Figure 4 presents the predicted amino acid sequence of DSP-13 (SEQ ID NO:6).

Figure 5 shows a cDNA sequence for a DSP-13 alternate splice variant (SEQ ID NO:7), with the start and stop codons indicated in bold (Fig. 5A); Fig. 5B shows the predicted amino acid sequence (SEQ ID NO:8) of the DSP-13 alternate splice variant encoded by SEQ ID NO 7.

Figure 6 is a sequence alignment showing sequence similarity between DSP-12, DSP-13 and other MAP-kinase phosphatases.

Figure 7 is a sequence alignment of full length DSP-12 (SEQ ID NO:2) and DSP-13 (SEQ ID NO:6) amino acid sequences.

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Detailed Description of the Invention

As noted above, the present invention is generally directed to compositions and methods for modulating (*i.e.*, stimulating or inhibiting) cellular proliferative responses, *in vitro* and *in vivo*. In particular, the present invention provides dual-specificity phosphatases DSP-12 (Figures 1-2; SEQ ID NOS:1-2) and DSP-13 (Figures 3-4; SEQ ID NOS:5-6), as well as variants thereof and antibodies that specifically bind DSP-12 or DSP-13. Also provided herein are methods for using such compounds for screens, detection assays and related therapeutic uses.

25 DSP-12 AND DSP-13 POLYPEPTIDES AND POLYNUCLEOTIDES

As used herein, the term "DSP-12 polypeptide" refers to a polypeptide that comprises a DSP-12 sequence as provided herein or a variant of such a sequence.

As used herein, the term "DSP-13 polypeptide" refers to a polypeptide that comprises a DSP-13

or a DSP-13 substrate, with an activity that is not substantially diminished relative to that of a full length native DSP-12 or DSP-13. DSP-12 substrates and DSP-13 substrates include activated (*i.e.*, phosphorylated) MAP-kinases. Other substrates may be identified using substrate trapping mutants, as described herein, and include polypeptides having one or more phosphorylated tyrosine, threonine and/or serine residues.

DSP-12 polypeptide variants and DSP-13 polypeptide variants within the scope of the present invention may contain one or more substitutions, deletions, additions and/or insertions. For certain DSP-12 and/or DSP-13 variants, the ability of the variant to dephosphorylate tyrosine and threonine residues within a DSP-12 substrate or a DSP-13 substrate is not substantially diminished. The ability of such a DSP-12 variant or a DSP-13 variant to dephosphorylate tyrosine and threonine residues within a DSP-12 substrate or a DSP-13 substrate may be enhanced or unchanged, relative to a native DSP-12 or a native DSP-13, or may be diminished by less than 50%, and preferably less than 20%, relative to native DSP-12 or DSP-13. Such variants may be identified using the representative assays provided herein.

Also contemplated by the present invention are modified forms of DSP-12 and/or DSP-13 in which a specific function is disabled. For example, such proteins may be constitutively active or inactive, or may display altered binding or catalytic properties. Such altered proteins may be generated using well known techniques, and the altered function confirmed using screens such as those provided herein. Certain modified DSP-12 and/or DSP-13 polypeptides are known as "substrate trapping mutants." Such polypeptides retain the ability to bind a substrate (*i.e.*, K_d is not substantially diminished), but display a reduced ability to dephosphorylate a substrate (*i.e.*, k_p is reduced, preferably to less than 1 per minute). Further, the stability of the substrate trapping mutant substrate complex should not be substantially diminished, relative to the stability of a DSP-12 substrate complex or a DSP-13 substrate complex. Complex stability may be assessed based on the association constant (K_d).

Worth Publishers, NY) and assays provided herein. Substrate trapping mutants may be generated, for example, by modifying DSP-12 with an amino acid substitution at position 222 or position 253 (e.g., by replacing the amino acid aspartate at position 222 with an alanine residue, or by replacing the cysteine at residue 253 with a serine).

5 Substrate trapping mutants may be generated, for example, by modifying DSP-13 with an amino acid substitution at position 368 or position 399 (e.g., by replacing the amino acid aspartate at position 368 with an alanine residue, or by replacing the cysteine at residue 399 with a serine). Substrate trapping mutants may be used, for example, to identify DSP-12 substrates or DSP-13 substrates. Briefly, the modified DSP-12 or

10 DSP-13 may be contacted with a candidate substrate (alone or within a mixture of proteins, such as a cell extract) to permit the formation of a substrate DSP-12 complex or a substrate DSP-13 complex. The complex may then be isolated by conventional techniques to permit the isolation and characterization of substrate. The preparation and use of substrate trapping mutants is described, for example, within PCI Publication

15 No. WO 98 04712.

Preferably, a variant contains conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydrophobic nature of the

20 polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups

25 having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr, (2) val, leu, ile, met, thr, (3) asp, glu, gln, asn, and (4) ala, val, leu, phe, met, tyr, asp, glu, gln, asn, and ser.

In general, modifications may be more readily made in non-critical regions, which are regions of the native sequence that do not substantially change the activity of DSP-12 or DSP-13. Non-critical regions may be identified by modifying the DSP-12 or DSP-13 sequence in a particular region and assaying the ability of the resulting variant in a phosphatase assay, as described herein. Preferred sequence modifications are made so as to retain the active site domain (CLVHCKMGVSRSAIVEAYAM; SEQ ID NO:3). Within certain preferred embodiments, such modifications affect interactions between DSP-12 or DSP-13 and cellular components other than DSP-12 substrates or DSP-13 substrates. However, substitutions may also be made in critical regions of the native protein, provided that the resulting variant substantially retains the ability to stimulate substrate dephosphorylation. Within certain embodiments, a variant contains substitutions, deletions, additions and/or insertions at no more than 50%, preferably no more than 25%, of the amino acid residues.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the activity of the polypeptide. In particular, variants may contain additional amino acid sequences at the amino and/or carboxy termini. Such sequences may be used, for example, to facilitate purification or detection of the polypeptide.

DSP-12 and/or DSP-13 polypeptides may be prepared using any of a variety of well known techniques. Recombinant polypeptides encoded by DNA sequences as described below may be readily prepared from the DNA sequences using any of a variety of expression vectors known to those of ordinary skill in the art. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells, and forms that differ in glycosylation may be generated by varying the

concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

Portions and other variants having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See Merrifield, *J. Am. Chem. Soc.* 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied BioSystems, Inc. (Foster City, CA), and may be operated according to the manufacturer's instructions.

A "DSP-12 polynucleotide" is any polynucleotide that encodes at least a portion of a DSP-12 polypeptide or a variant thereof, or that is complementary to such a polynucleotide. A "DSP-13 polynucleotide" is any polynucleotide that encodes at least a portion of a DSP-13 polypeptide or a variant thereof, or that is complementary to such a polynucleotide. Preferred polynucleotides comprise at least 15 consecutive nucleotides, preferably at least 30 consecutive nucleotides, that encode a DSP-12 polypeptide or a DSP-13 polypeptide or that are complementary to such a sequence. Certain polynucleotides encode a DSP-12 polypeptide or a DSP-13 polypeptide; others may find use as probes, primers or antisense oligonucleotides, as described below. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

DSP-12 polynucleotides and/or DSP-13 polynucleotides may comprise a native sequence or a modified sequence. DSP-12 or DSP-13 sequences may be modified

the activity of the encoded polypeptide is not substantially diminished, as described above. The effect on the activity of the encoded polypeptide may generally be assessed as described herein. Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native DSP-12 or a portion thereof, or to a polynucleotide sequence that encodes a native DSP-13 or a portion thereof. The percent identity may be readily determined by comparing sequences using computer algorithms well known to those of ordinary skill in the art, such as Align or the BLAST algorithm (Altschul, *J. Mol. Biol.* 219:555-565, 1991; Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915-10919, 1992), which is available at the NCBI website (<http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST>). Default parameters may be used. Certain variants are substantially homologous to a native gene. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA or RNA sequence encoding a native DSP-12 (or a complementary sequence) or a native DSP-13 (or a complementary sequence). Suitable moderately stringent conditions include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50 °C-65 °C, 5 X SSC, overnight; followed by washing twice at 65 °C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. For additional stringency, conditions may include a wash in 0.1X SSC and 0.1% SDS at 60 °C for 15 minutes.

It will be appreciated by those having ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention.

Polynucleotides may be prepared using any of a variety of techniques. For example, a polynucleotide may be amplified from cDNA prepared from a suitable

approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

An amplified portion may be used to isolate a full length gene from a suitable library (e.g., human brain, testis, kidney, liver or skeletal muscle cDNA) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (e.g., by nick-translation or end-labeling with ^{32}P) using well known techniques. A bacterial or bacteriophage library may then be screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (see Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. Clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. One such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer, which hybridizes to a poly A region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Any of a variety of commercially available kits may be used to perform the construction of a RACE

the target sequence at temperatures of about 54 °C to 72 °C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

A cDNA sequence encoding DSP-12 is provided in Figure 1 (SEQ ID NO:1), and the predicted amino acid sequence is provided in Figure 2 (SEQ ID NO:2). The DSP-12 active site (CIVHCKMGVSRSAIVLAYAM; SEQ ID NO:3) is located at positions 249 through 269 of SEQ ID NO:2 (Figure 7). Sequence information immediately adjacent to this site was used to design 5' and 3' RACE reactions with human skeletal muscle and testis cDNA to identify a 1,656 base pair cDNA that corresponds to a mRNA that is ubiquitously expressed in a variety of tissues examined. This cDNA encodes a protein of 552 amino acids that is referred to herein as DSP-12. DSP-12 shows significant homology to other MAP-kinase phosphatases, as shown by the sequence comparison presented in Figure 6.

A cDNA sequence encoding DSP-13 is provided in Figure 3 (SEQ ID NO:5), and the predicted amino acid sequence is provided in Figure 4 (SEQ ID NO:6). The DSP-13 active site (CIVHCKMGVSRSAIVLAYAM; SEQ ID NO:3), is located at positions 395 through 415 of SEQ ID NO:6 (Figure 7). Sequence information immediately adjacent to this site was used to design 5' and 3' RACE reactions with human skeletal muscle and testis cDNA to identify a 1,527 base pair cDNA that corresponds to a mRNA that is ubiquitously expressed in a variety of tissues examined. This cDNA encodes a protein of 509 amino acids that is referred to herein as DSP-13. DSP-13 shows significant homology to other MAP-kinase phosphatases, as shown by the sequence comparison presented in Figure 6. An alternate splice variant of DSP-1 is encoded by a 723 base pair cDNA, which encodes a protein of 241 amino acids (Figure 5).

DSP-12 polynucleotide variants and DSP-13 polynucleotide variants may generally be prepared by any method known in the art, including, for example, solid phase chemical synthesis. An additional embodiment of the present invention

or *in vivo* transcription of DNA sequences encoding DSP-12 and or DSP-13, or portion thereof, provided that the DNA is incorporated into a vector with a suitable RNA polymerase promoter (such as T7 or SP6). Certain polynucleotides may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a polynucleotide may be administered to a patient such that the encoded polypeptide is generated *in vivo*.

A polynucleotide that is complementary to at least a portion of a coding sequence (e.g., an antisense polynucleotide or a ribozyme) may also be used as a probe or primer, or to modulate gene expression. Identification of oligonucleotides and ribozymes for use as antisense agents, and DNA encoding genes for their targeted delivery, involve methods well known in the art. For example, the desirable properties, lengths and other characteristics of such oligonucleotides are well known. Antisense oligonucleotides are typically designed to resist degradation by endogenous nucleolytic enzymes by using such linkages as: phosphorothioate, methylphosphonate, sulfone, sulfate, ketyl, phosphorodithioate, phosphoramidate, phosphate esters, and other such linkages (see, e.g., Agrwal et al., *Tetrahedron Lett.* 28:3539-3542 (1987); Miller et al., *J. Am. Chem. Soc.* 93:6657-6665 (1971); Stee et al., *Tetrahedron Lett.* 26:2191-2194 (1985); Moody et al., *Nucl. Acids Res.* 12:4769-4782 (1989); Uznanski et al., *Nucl. Acids Res.* (1989); Letsinger et al., *Tetrahedron* 40:137-143 (1984); Eckstein, *Annu. Rev. Biochem.* 54:367-402 (1985); Eckstein, *Trends Biol. Sci.* 14:97-100 (1989); Stein, In: *Oligodeoxynucleotides, Antisense Inhibitors of Gene Expression*, Cohen, Ed., Macmillan Press, London, pp. 97-117 (1989); Jager et al., *Biochemistry* 27:7237-7246 (1988)).

Antisense polynucleotides are oligonucleotides that bind in a sequence-specific manner to nucleic acids, such as mRNA or DNA. When bound to mRNA that has complementary sequences, antisense prevents translation of the mRNA (see, e.g., U.S. Patent No. 5,168,053 to Altman et al.; U.S. Patent No. 5,190,931 to Inouye; U.S. Patent No. 5,135,917 to Ruedy; U.S. Patent No. 5,087,617 to Saito et al.; U.S. Patent

DNA forming a colinear triplex molecule, thereby preventing transcription (see, e.g., U.S. Patent No. 5,176,996 to Hogan et al., which describes methods for making synthetic oligonucleotides that bind to target sites on duplex DNA).

Particularly useful antisense nucleotides and triplex molecules are molecules that are complementary to or bind the sense strand of DNA or mRNA that encodes a DSP-12 polypeptide or a DSP-13 polypeptide or a protein mediating any other process related to expression of endogenous DSP-12 and/or DSP-13, such that inhibition of translation of mRNA encoding the DSP-12 polypeptide or the DSP-13 polypeptide is affected. cDNA constructs that can be transcribed into antisense RNA may also be introduced into cells or tissues to facilitate the production of antisense RNA. Antisense technology can be used to control gene expression through interference with binding of polymerases, transcription factors or other regulatory molecules (see Gee et al., *In Huber and Carr, Molecular and Immunologic Approaches*, Futura Publishing Co. (Mt. Kisco, NY: 1994)). Alternatively, an antisense molecule may be designed to hybridize with a control region of a DSP-12 gene or a DSP-13 gene (e.g., promoter, enhancer or transcription initiation site), and block transcription of the gene; or to block translation by inhibiting binding of a transcript to ribosomes.

The present invention also contemplates DSP-12-specific and DSP-13-specific ribozymes. A ribozyme is an RNA molecule that specifically cleaves RNA substrates, such as mRNA, resulting in specific inhibition or interference with cellular gene expression. There are at least five known classes of ribozymes involved in the cleavage and/or ligation of RNA chains. Ribozymes can be targeted to any RNA transcript and can catalytically cleave such transcripts (see, e.g., U.S. Patent No. 5,272,262; U.S. Patent No. 5,144,019; and U.S. Patent Nos. 5,168,053, 5,180,818, 5,116,742 and 5,093,246 to Cech et al.). Any DSP-12 mRNA-specific ribozyme or DSP-13 mRNA-specific ribozyme, or a nucleic acid encoding such a ribozyme, may be delivered to a host cell to effect inhibition of DSP-12 or DSP-13 gene expression. Ribozymes may therefore be delivered to the host cells by DNA encoding the ribozyme.

Any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl-, methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a suitable vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Other elements will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

Within certain embodiments, polynucleotides may be formulated so as to permit entry into a cell of a mammal, and expression therein. Such formulations are particularly useful for therapeutic purposes, as described below. Those of ordinary skill in the art will appreciate that there are many ways to achieve expression of a polynucleotide in a target cell, and any suitable method may be employed. For example, a polynucleotide may be incorporated into a viral vector using well known techniques. A viral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target specific. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

Other formulations for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microparticles, beads, or

in vivo is a liposome (*i.e.*, an artificial membrane vesicle). The preparation and use of such systems is well known in the art.

Within other aspects, a DSP-12 promoter or a DSP-13 promoter may be isolated using standard techniques. The present invention provides nucleic acid molecules comprising such a promoter or one or more *cis*- or *trans*-acting regulatory elements thereof. Such regulatory elements may enhance or suppress expression of DSP-12 or DSP-13. A 5' flanking region may be generated using standard techniques, based on the genomic sequence provided herein. If necessary, additional 5' sequences may be generated using PCR-based or other standard methods. The 5' region may be subcloned and sequenced using standard methods. Primer extension and/or RNase protection analyses may be used to verify the transcriptional start site deduced from the cDNA.

To define the boundary of the promoter region, putative promoter inserts of varying sizes may be subcloned into a heterologous expression system containing a suitable reporter gene without a promoter or enhancer. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase or the Green Fluorescent Protein gene. Suitable expression systems are well known and may be prepared using well known techniques or obtained commercially. Internal deletion constructs may be generated using unique internal restriction sites or by partial digestion of non-unique restriction sites. Constructs may then be transfected into cells that display high levels of DSP-12 and/or DSP-13 expression. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate DSP-12 and/or DSP-13 transcription.

Once a functional promoter is identified, *cis*- and *trans*-acting elements may be located. *Cis*-acting sequences may generally be identified based on homology to previously characterized transcriptional motifs. Point mutations, deletions, or

techniques or a PCR-based strategy. The altered promoter is then cloned into a reporter gene expression vector, as described above, and the effect of the mutation on reporter gene expression is evaluated.

The present invention also contemplates the use of allelic variants of DSP-12 and DSP-13, as well as DSP-12 and DSP-13 sequences from other organisms. Such sequences may generally be identified based upon similarity to the sequences provided herein (e.g., using hybridization techniques) and based upon the presence of DSP-12 activity and/or DSP-13 activity, using an assay provided herein.

In general, polypeptides and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

ASSAYS FOR DETECTING DSP-12 AND DSP-13 ACTIVITY

DSP-12 polypeptide variants may be tested for DSP-12 activity, and DSP-13 polypeptide variants may be tested for DSP-13 activity, using any suitable assay for MAP-kinase phosphatase activity. Such assays may be performed *in vitro* or within a cell-based assay. For example, a MAP-kinase may be obtained in inactive form from Upstate Biotechnology (Lake Placid, NY; catalog number 14-198). Phosphorylation of the MAP-kinase can be performed using well known techniques (such as those described by Zheng and Guan, *J. Biol. Chem.* 268:16116-16119, 1993) using the MAP-kinase kinase MEK-1 (available from Upstate Biotechnology; cat. no. 14-206). Radiolabeled substrate may be used for the kinase reaction, resulting in radiolabeled γ -phospho-MAP-kinase. A DSP-12 or DSP-13 will catalyze the DSP-12 or DSP-13

conditions (e.g., Tris, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 1 mg/ml bovine serum albumin for 10 minutes at 30 °C) or as described by Zheng and Guan, *J. Biol. Chem.* 268:16116-16119, 1993). Dephosphorylation of the MAP-kinase may be detected using any of a variety of assays, such as a coupled kinase assay (evaluating phosphorylation of a MAP-kinase substrate using any assay generally known in the art) or directly, based on (1) the loss of radioactive phosphate groups (e.g., by gel electrophoresis, followed by autoradiography); (2) the shift in electrophoretic mobility following dephosphorylation; (3) the loss of reactivity with an antibody specific for phosphotyrosine or phosphothreonine; or (4) a phosphoamino acid analysis of the MAP-kinase. Certain assays may generally be performed as described by Ward et al., *Nature* 367:651-654, 1994 or Alessi et al., *Oncogene* 8:2015-2020, 1993. In general, contact of 500 pg - 50 ng of DSP-12 or DSP-13 polypeptide with 100ng - 100 µg activated MAP-kinase should result in a detectable dephosphorylation of the MAP-kinase, typically within 20-30 minutes. Within certain embodiments, 0.01 - 10 units/ml (preferably about 0.1 units/ml, where a unit is an amount sufficient to dephosphorylate 1 nmol substrate per minute) DSP-12 or DSP-13 polypeptide may be contacted with 0.1 - 10 µM (preferably about 1 µM) activated MAP-kinase to produce a detectable dephosphorylation of a MAP-kinase. Preferably, a DSP-12 or DSP-13 polypeptide results in dephosphorylation of a MAP-kinase or a phosphorylated substrate (such as a tyrosine- and/or serine-phosphorylated peptide) that is at least as great as the dephosphorylation observed in the presence of a comparable amount of native human DSP-12 or DSP-13. It will be apparent that other substrates identified using a substrate trapping mutant as described herein may be substituted for the MAP-kinase within such assays.

25

ANTIBODIES AND ANTIGEN-BINDING FRAGMENTS

Also contemplated by the present invention are peptides, polypeptides and other non-peptide molecules that cross-react with DSP-12 or DSP-13.

unrelated sequence or a sequence of a different dual-specificity phosphatase. Similarly, a molecule is said to "specifically bind" to DSP-13 if it reacts at a detectable level with DSP-13, and does not react detectably with peptides containing an unrelated sequence or a sequence of a different dual-specificity phosphatase. Such binding properties may generally be assessed using an ELISA, which may be readily performed by those of ordinary skill in the art. Preferred binding molecules include antibodies (which may be, for example, polyclonal, monoclonal, single chain, chimeric, anti-idiotypic or CDR-grafted). Certain preferred antibodies are those antibodies that inhibit or block DSP-12 and or DSP-13 activity within an *in vitro* assay, as described herein.

Antibodies may generally be prepared by any of a variety of techniques using isolated native or recombinant DSP-12 protein, or recombinant DSP-13 protein, as antigen. Such techniques are known to those having ordinary skill in the art (see, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988). In one such technique, an immunogen comprising a DSP-12 polypeptide (or a DSP-13 polypeptide) is initially injected into a suitable animal (e.g., mice, rats, rabbits, sheep and goats), preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for DSP-12 or a variant thereof, or for DSP-13 or a variant thereof, may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is

low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity
5 against the polypeptide. Hybridomas having high reactivity and specificity are preferred. Hybridomas that generate monoclonal antibodies that specifically bind to DSP-12 (or that specifically bind to DSP-13) are contemplated by the present invention.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the
10 yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction.

15 Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques (e.g., by digestion with papain to yield Fab and Fc fragments). The Fab and Fc fragments may be separated by affinity chromatography (e.g., on protein A bead columns), using standard techniques.

20 Polyclonal and monoclonal antibodies may be used for the affinity isolation of DSP-12 or DSP-13 polypeptides. Techniques for affinity purification of a polypeptide are well known in the art (see, e.g., Hermanson, G. L., et al., "Immobilized Affinity Ligand Techniques," Academic Press, Inc. (New York, 1992)). Briefly, an antibody or antigen-binding fragment thereof may be immobilized on a solid support
25 material, which is then contacted with a sample comprising the polypeptide of interest. Following separation from the remainder of the sample, the polypeptide is then released from the immobilized antibody.

METHODS FOR DETECTING DSP-12 AND DSP-13 EXPRESSION

Certain aspects of the present invention provide methods that employ antibodies raised against DSP-12 or DSP-13, or hybridizing polynucleotides, for diagnostic and assay purposes. Certain assays involve using an antibody or other agent to detect the presence or absence of DSP-12 or DSP-13, or proteolytic fragments thereof. Alternatively, nucleic acid encoding DSP-12 or DSP-13 may be detected, using standard hybridization and or PCR techniques. Suitable probes and primers may be designed by those having ordinary skill in the art based on the DSP-12 and DSP-13 cDNA sequences provided herein. Assays may generally be performed using any of a variety of samples obtained from a biological source, such as eukaryotic cells, bacteria, viruses, extracts prepared from such organisms and fluids found within living organisms. Biological samples that may be obtained from a patient include blood samples, biopsy specimens, tissue explants, organ cultures and other tissue or cell preparations. A patient or biological source may be a human or non-human animal, a primary cell culture or culture adapted cell line including but not limited to genetically engineered cell lines that may contain chromosomally integrated or episomal recombinant nucleic acid sequences, immortalized or immortalizable cell lines, somatic cell hybrid cell lines, differentiated or differentiable cell lines, transformed cell lines and the like. In certain preferred embodiments of the invention, a patient may be suspected of having or being at risk for having a disease associated with altered cellular signal transduction, or may be known to be free of a risk for or presence of such as disease.

To detect DSP-12 protein or DSP-13 protein, the reagent is typically an antibody, which may be prepared as described below. There are a variety of assay formats known to those having ordinary skill in the art for using an antibody to detect a polypeptide in a sample. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory, 1988). For example, the assay may be

allowed to react with the antibody. The presence of the antibody on the membrane may then be detected using a suitable detection reagent, as described below.

In another embodiment, the assay involves the use of antibody immobilized on a solid support to bind to the target DSP-12 (or DSP-13) and remove it from the remainder of the sample. The bound DSP-12 (or DSP-13) may then be detected using a second antibody or reagent that contains a reporter group. Alternatively, a competitive assay may be utilized, in which a DSP-12 or DSP-13 polypeptide is labeled with a reporter group and allowed to bind to the immobilized antibody after incubation of the antibody with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the antibody is indicative of the reactivity of the sample with the immobilized antibody, and as a result, indicative of the level of DSP-12 or DSP-13 in the sample.

The solid support may be any material known to those of ordinary skill in the art to which the antibody may be attached, such as a test well in a microtiter plate, a nitrocellulose filter or another suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic such as polystyrene or polyvinylchloride. The antibody may be immobilized on the solid support using a variety of techniques known to those in the art, which are amply described in the patent and scientific literature.

In certain embodiments, the assay for detection of DSP-12 or DSP-13 in a sample is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the biological sample, such that DSP-12 (or DSP-13) within the sample is allowed to bind to the immobilized antibody (a 30 minute incubation time at room temperature is generally sufficient). Unbound sample is then removed from the immobilized DSP-12 antibody complexes (or DSP-13 antibody complexes) and a second antibody (containing a reporter group such as an enzyme, dye, radionuclide, luminescent group, fluorescent group or biotin) capable of binding to

the specific reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products. Standards and standard additions may be used to determine the level of DSP-12 or DSP-13 in a sample, using well known techniques.

In a related aspect of the present invention, kits for detecting DSP-12 (or DSP-13) and DSP-12 (or DSP-13) phosphatase activity are provided. Such kits may be designed for detecting the level of DSP-12 (or DSP-13) or nucleic acid encoding DSP-12 (or DSP-13), or may detect phosphatase activity of DSP-12 (or DSP-13) in a direct phosphatase assay or a coupled phosphatase assay. In general, the kits of the present invention comprise one or more containers enclosing elements, such as reagents or buffers, to be used in the assay.

A kit for detecting the level of DSP-12 (or DSP-13), or nucleic acid encoding DSP-12 (or DSP-13), typically contains a reagent that binds to the DSP-12 (or DSP-13) protein, DNA or RNA. To detect nucleic acid encoding DSP-12 (or DSP-13), the reagent may be a nucleic acid probe or a PCR primer. To detect DSP-12 or DSP-13 protein, the reagent is typically an antibody. Such kits also contain a reporter group suitable for direct or indirect detection of the reagent (*i.e.* the reporter group may be covalently bound to the reagent or may be bound to a second molecule, such as Protein A, Protein G, immunoglobulin or lectin, which is itself capable of binding to the reagent). Suitable reporter groups include, but are not limited to, enzymes (e.g., horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. Such reporter groups may be used to directly or indirectly detect binding of the reagent to a sample, such as a cell or

Kits for detecting DSP-12 activity typically comprise a DSP-12 substrate in combination with a suitable buffer; similarly, kits for detecting DSP-13 activity typically comprise a DSP-13 substrate in combination with a suitable buffer. DSP-12 activity or DSP-13 activity may be specifically detected by performing an immunoprecipitation step with a DSP-12-specific (or a DSP-13-specific) antibody prior to performing a phosphatase assay as described above. Other reagents for use in detecting dephosphorylation of substrate may also be provided.

Within certain diagnostic assays, a proliferative disorder may be detected in a patient based on the presence of an altered DSP-12 or DSP-13, or an altered level of DSP-12 expression or of DSP-13 expression. For example, an antibody may distinguish between a wild-type DSP-12 and an altered DSP-12 having a variation in amino acid sequence, or between a wild-type DSP-13 and an altered DSP-13 having a variation in amino acid sequence. Such a variation may be indicative of the presence of a proliferative disorder, or of susceptibility to such a disorder. Hybridization and amplification techniques may be similarly used to detect modified DSP-12 or DSP-13 sequences.

METHODS FOR IDENTIFYING MODULATORS OF DSP-12 AND DSP-13 ACTIVITY

In one aspect of the present invention, DSP-12 or DSP-13 polypeptides may be used to identify agents that modulate DSP activity. Such agents may inhibit or enhance signal transduction via a MAP-kinase cascade, leading to cell proliferation. An agent that modulates DSP-12 or DSP-13 activity may alter expression and/or stability of DSP-12 or DSP-13, DSP-12 or DSP-13 protein activity and/or the ability of DSP-12 or DSP-13 to dephosphorylate a substrate. Agents that may be screened within such assays include, but are not limited to, antibodies and antigen-binding fragments thereof, competing peptides that represent, for example, a catalytic site or a dual phosphorylation motif, antisense polynucleotides and ribozymes that interfere with transcription and/or translation of DSP-12 or DSP-13, and other natural and synthetic

Candidate agents for use in a method of screening for a modulator of DSP-12 or DSP-13 according to the present invention may be provided as "libraries" or collections of compounds, compositions or molecules. Such molecules typically include compounds known in the art as "small molecules" and having molecular weights less than 10^4 daltons, preferably less than 10^3 daltons and still more preferably less than 10^2 daltons. For example, members of a library of test compounds can be administered to a plurality of samples, each containing at least one DSP-12 or DSP-13 polypeptide as provided herein, and then assayed for their ability to enhance or inhibit DSP-12- or DSP-13-mediated dephosphorylation of, or binding to, a substrate. Compounds so identified as capable of influencing DSP-12 or DSP-13 function (e.g., phosphotyrosine and/or phosphoserine/threonine dephosphorylation) are valuable for therapeutic and/or diagnostic purposes, since they permit treatment and/or detection of diseases associated with DSP-12 or DSP-13 activity. Such compounds are also valuable in research directed to molecular signaling mechanisms that involve DSP-12 or DSP-13, and to refinements in the discovery and development of future DSP-12 or DSP-13 compounds exhibiting greater specificity.

Candidate agents further may be provided as members of a combinatorial library, which preferably includes synthetic agents prepared according to a plurality of predetermined chemical reactions performed in a plurality of reaction vessels. For example, various starting compounds may be prepared employing one or more of solid-phase synthesis, recorded random mix methodologies and recorded reaction split techniques that permit a given constituent to traceably undergo a plurality of permutations and/or combinations of reaction conditions. The resulting products comprise a library that can be screened followed by iterative selection and synthesis procedures, such as a synthetic combinatorial library of peptides (see e.g., PCT US91/08694, PCT US91/04666, which are hereby incorporated by reference in their entirety) or other compositions that may include small molecules as provided herein (see e.g., PCT US94/08542, EP 0774464, U.S. 5,798,035, U.S. 5,780,173, U.S.

may be prepared according to established procedures, and tested using DSP-12 and or DSP-13 according to the present disclosure.

In certain embodiments, modulating agents may be identified by combining a candidate agent with a DSP-12 or DSP-13 polypeptide or a polynucleotide encoding such a polypeptide, *in vitro* or *in vivo*, and evaluating the effect of the candidate agent on the DSP-12 or DSP-13 phosphatase activity using, for example, a representative assay described herein. An increase or decrease in phosphatase activity can be measured by performing a representative assay provided herein in the presence and absence of a candidate agent. Briefly, a candidate agent may be included in a mixture of active DSP-12 or DSP-13 polypeptide and substrate (e.g., a phosphorylated MAP-kinase), with or without pre-incubation with one or more components of the mixture. In general, a suitable amount of antibody or other agent for use in such an assay ranges from about 0.01 μ M to about 100 μ M. The effect of the agent on DSP-12 or DSP-13 activity may then be evaluated by quantitating the loss of phosphate from the substrate, and comparing the loss with that achieved using DSP-12 or DSP-13 without the addition of a candidate agent. Alternatively, a coupled kinase assay may be used, in which DSP-12 or DSP-13 activity is indirectly measured based on MAP-kinase activity.

Alternatively, a polynucleotide comprising a DSP-12 promoter operably linked to a DSP-12 coding region or reporter gene may be used to evaluate the effect of a test compound on DSP-12 transcription. Similarly, a polynucleotide comprising a DSP-13 promoter operably linked to a DSP-13 coding region or reporter gene may be used to evaluate the effect of a test compound on DSP-13 transcription. Such assays may be performed in cells that express DSP-12 or DSP-13 endogenously (e.g., human kidney or liver) or in cells transfected with an expression vector comprising a DSP-12 promoter or a DSP-13 promoter linked to a reporter gene. The effect of a test compound may then be evaluated by assaying the effect on transcription of DSP-12 (or DSP-13) or the reporter using, for example, a Northern blot analysis or a suitable reporter activity assay.

an appropriate substrate. For example, appropriate cells (*i.e.*, cells that express DSP-12 or DSP-13) may be transfected with a substrate-dependent promoter linked to a reporter gene. In such a system, expression of the reporter gene (which may be readily detected using methods well known to those of ordinary skill in the art) depends upon activation of substrate. Dephosphorylation of substrate may be detected based on a decrease in reporter activity. Candidate modulating agents may be added to such a system, as described above, to evaluate their effect on DSP-12 or DSP-13 activity.

The present invention further provides methods for identifying a molecule that interacts with, or binds to, DSP-12 or DSP-13. Such a molecule generally associates with DSP-12 or DSP-13 with an affinity constant (K_d) of at least 10^4 , preferably at least 10^5 , more preferably at least 10^6 , still more preferably at least 10^7 and most preferably at least 10^8 . Affinity constants may be determined using well known techniques. Methods for identifying interacting molecules may be used, for example, as initial screens for modulating agents, or to identify factors that are involved in the *in vivo* DSP-12 or DSP-13 activity. Techniques for substrate trapping of DSP-12 or DSP-13 variants are also included. In addition to standard binding assays, there are many other techniques that are well known for identifying interacting molecules, including yeast two-hybrid screens, phage display and affinity techniques. Such techniques may be performed using routine protocols, which are well known to those of ordinary skill in the art (see, e.g., Bartel et al., In *Cellular Interactions in Development: A Practical Approach*, D.A. Harley, ed., Oxford University Press (Oxford, UK), pp. 153-179, 1993). Within these and other techniques, proteins may be phosphorylated prior to assaying for interacting proteins.

Within other aspects, the present invention provides animal models in which an animal either does not express a functional DSP-12 (or DSP-13), or expresses an altered DSP-12 (or DSP-13). Such animals may be generated using standard homologous recombination strategies. Animal models generated in this manner may be used to study activities of DSP-12 or DSP-13 polypeptides, and modulating agents, or

METHODS FOR DEPHOSPHORYLATING A SUBSTRATE

In another aspect of the present invention, a DSP-12 polypeptide may be used for dephosphorylating a substrate of DSP-12, or a DSP-13 polypeptide may be used for dephosphorylating a substrate of DSP-13. In one embodiment, a substrate may be dephosphorylated *in vitro* by incubating a DSP-12 or DSP-13 polypeptide with a substrate in a suitable buffer (e.g., Tris, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 1 mg/ml bovine serum albumin) for 10 minutes at 30 °C. Any compound that can be dephosphorylated by DSP-12 or DSP-13, such as a MAP-kinase, may be used as a substrate. In general, the amounts of the reaction components may range from about 50 pg to about 50 ng of DSP-12 or DSP-13 polypeptide and from about 10 ng to about 10 µg of substrate. Dephosphorylated substrate may then be purified, for example, by affinity techniques and/or gel electrophoresis. The extent of substrate dephosphorylation may generally be monitored by adding [γ - 32 P]labeled substrate to a test aliquot, and evaluating the level of substrate dephosphorylation as described herein.

METHODS FOR MODULATING CELLULAR RESPONSES

Modulating agents may be used to modulate cellular responses such as cell proliferation, differentiation and survival, in a variety of contexts, both *in vivo* and *in vitro*. In general, to modulate such a response, a cell is contacted with an agent that modulates DSP-12 or DSP-13 activity, under conditions and for a time sufficient to permit modulation of DSP-12 or DSP-13 activity. Agents that modulate a cellular response may function in any of a variety of ways. For example, an agent may modulate a pattern of gene expression (i.e., may enhance or inhibit expression of a family of genes or genes that are expressed in a coordinated fashion). A variety of hybridization and amplification techniques are available for evaluating patterns of gene expression. Alternatively, or in addition, an agent may affect apoptosis or necrosis of the cell, and/or may modulate the functioning of the cell cycle within the cell. (See, e.g., Ashkenazi et al., 1998, *Science*, 281:1305; Thornberry et al., 1998, *Science*,

Cells treated as described above may display standard characteristics of cells with altered proliferation, differentiation or survival. In addition, such cells may (but need not) display other detectable properties, such as contact inhibition of cell growth, anchorage independent growth or altered intercellular adhesion. Such properties may be detected using techniques that are well known in the art.

THERAPEUTIC METHODS

One or more DSP-12 or DSP-13 polypeptides, modulating agents and or polynucleotides encoding such polypeptides and or modulating agents may also be used to modulate DSP-12 or DSP-13 activity in a patient. As used herein, a "patient" may be any mammal, including a human, and may be afflicted with a condition associated with DSP-12 or DSP-13 activity or may be free of detectable disease. Accordingly, the treatment may be of an existing disease or may be prophylactic. Conditions associated with DSP-12 or DSP-13 activity include any disorder associated with cell proliferation, including Duchenne muscular dystrophy, cancer, graft-versus-host disease (GVHD), autoimmune diseases, allergy or other conditions in which immunosuppression may be involved, metabolic diseases, abnormal cell growth or proliferation and cell cycle abnormalities. Certain such disorders involve loss of normal MAP-kinase phosphatase activity, leading to uncontrolled cell growth. DSP-12 or DSP-13 polypeptides, and polynucleotides encoding such polypeptides, can be used to ameliorate such disorders.

For administration to a patient, one or more polypeptides, polynucleotides and or modulating agents are generally formulated as a pharmaceutical composition. A pharmaceutical composition may be a sterile aqueous or non-aqueous solution, suspension or emulsion, which additionally comprises a physiologically acceptable carrier (*i.e.* a non-toxic material that does not interfere with the activity of the active ingredient). Such compositions may be in the form of a solid, liquid or gas (aerosol). Alternatively, compositions of the present invention may be formulated as a lyophilizate or compounds may be encapsulated within liposomes or in other well known

components include, but are not limited to, buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, mannose, sucrose or dextran), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, stabilizers, dyes, flavoring agents, and suspending agents and or preservatives.

Any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of the present invention. Carriers for therapeutic use are well known, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro ed. 1985). In general, the type of carrier is selected based on the mode of administration. Pharmaceutical compositions may be formulated for any appropriate manner of administration, including, for example, topical, oral, nasal, intrathecal, rectal, vaginal, sublingual or parenteral administration, including subcutaneous, intravenous, intramuscular, intrasternal, intracavernous, intrameatal or intraurethral injection or infusion. For parenteral administration, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, kaolin, glycerin, starch dextrins, sodium alginate, carboxymethylcellulose, ethyl cellulose, glucose, sucrose and or magnesium carbonate, may be employed.

A pharmaceutical composition (e.g., for oral administration or delivery by injection) may be in the form of a liquid (e.g., an elixir, syrup, solution, emulsion or suspension). A liquid pharmaceutical composition may include, for example, one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such

disposable syringes or multiple dose vials made of glass or plastic. The use of physiological saline is preferred, and an injectable pharmaceutical composition is preferably sterile.

The compositions described herein may be formulated for sustained release (*i.e.*, a formulation such as a capsule or sponge that effects a slow release of compound following administration). Such compositions may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain an agent dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

For pharmaceutical compositions comprising a polynucleotide encoding a DSP-12 or DSP-13 polypeptide and/or modulating agent (such that the polypeptide and/or modulating agent is generated *in situ*), the polynucleotide may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid, and bacterial, viral and mammalian expression systems. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ullmer et al., *Science*, 259:1745-1749, 1993 and reviewed by Cohen, *Science*, 259:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

Within a pharmaceutical composition, a DSP-12 or DSP-13 polypeptide, polynucleotide or modulating agent may be linked to any of a variety of compounds. For example, such an agent may be linked to a function modulating compound, such as

compound or cell) that, when linked to an agent enhances the transport of the agent to a target cell or tissue, thereby increasing the local concentration of the agent. Targeting moieties include antibodies or fragments thereof, receptors, ligands and other molecules that bind to cells of, or in the vicinity of, the target tissue. An antibody targeting agent may be an intact (whole) molecule, a fragment thereof, or a functional equivalent thereof. Examples of antibody fragments are F(ab')₂, F(ab)', Fab and Fv fragments, which may be produced by conventional methods or by genetic or protein engineering. Linkage is generally covalent and may be achieved by, for example, direct condensation or other reactions, or by way of bi- or multi-functional linkers. Targeting moieties may be selected based on the cell(s) or tissue(s) at which the agent is expected to exert a therapeutic benefit.

Pharmaceutical compositions may be administered in a manner appropriate to the disease to be treated (or prevented). An appropriate dosage and a suitable duration and frequency of administration will be determined by such factors as the condition of the patient, the type and severity of the patient's disease, the particular form of the active ingredient and the method of administration. In general, an appropriate dosage and treatment regimen provides the agent(s) in an amount sufficient to provide therapeutic and or prophylactic benefit (e.g., an improved clinical outcome, such as more frequent complete or partial remissions, or longer disease-free and or overall survival). For prophylactic use, a dose should be sufficient to prevent, delay the onset of or diminish the severity of a disease associated with cell proliferation.

Optimal dosages may generally be determined using experimental models and or clinical trials. In general, the amount of polypeptide present in a dose, or produced *in situ* by DNA present in a dose, ranges from about 0.01 µg to about 100 µg per kg of host, typically from about 0.1 µg to about 10 µg. The use of the minimum dosage that is sufficient to provide effective therapy is usually preferred. Patients may generally be monitored for therapeutic or prophylactic effectiveness using assays suitable for the condition being treated or prevented, which will be familiar to those of

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

Cloning and Sequencing cDNA Encoding DSP-12 and DSP-13

5 This Example illustrates the cloning of cDNA molecules encoding human DSP-12 and DSP-13.

A conserved sequence motif defining a novel homology domain of dual-specificity phosphatases was identified as follows: Dual specificity phosphatases belong to the larger family of protein tyrosine phosphatases (PTPs) that share a
 10 conserved catalytic domain containing a cysteine residue situated N-terminal to a stretch of five variable amino acids followed by an arginine residue (Fauman et al., *Trends In Bioch. Sci.* 21:413-417, 1996). DSPs typically contain a PTP active site motif but lack sequence homology to PTPs in other regions (Jia, *Biochem. and Cell Biol.* 75:17-26, 1997). There is, however, no reported consensus sequence that is
 15 conserved among DSPs, nor is a consensus region apparent from examination of the known DSP sequences such as those referred to above. To derive a longer consensus DSP amino acid sequence motif that would be useful for the identification of new DSP family members, multiple known human dual-specificity phosphatases sequences were aligned and compared. From an alignment of nine amino acid sequences derived from
 20 nine particular human DSPs having MAP-kinase phosphatase activity (Figure 6), a candidate conserved homology region was identified. This homology region consisted of a 24-amino acid peptide sequence, based on analysis of the DSP regions situated on either side of, and including, the PTP active site signature motif (including the conserved catalytic domain at positions 123-129 of Fig. 6). Thus, a candidate peptide
 25 having the sequence:

NGRAIVHCQAGSRSGENHAYTEM SEQ ID NO:1

search employed an algorithm (tblastn) capable of reverse translation of the candidate peptide with iterations allowing for genetic code degeneracy within default parameters. The search results identified a portion (nucleotides at positions numbers 54301 to 55500) of the HIGS entry having the accession number HIGS AC008081 as a candidate MAP-kinase phosphatase sequence encoding a MAP kinase phosphatase catalytic domain. HIGS AC008081 did not include a complete coding region of an expressed gene such as a gene encoding a DSP-12 having MAP-kinase phosphatase activity, nor were the sense strand and open reading frame identified.

To verify that the identified portion of the HIGS AC008081 sequence exists as an expressed sequence (*i.e.*, as transcribed mRNA) in tissues expressing DSP-12, RT-PCR was performed using human cDNA reverse transcribed from human skeletal muscle and testis RNA. The cDNA was screened in 5' and 3' RACE (rapid amplification of cDNA ends) reactions as described (Frohman et al., *Proc. Nat. Acad. Sci. USA* 85:8998, 1988; Ohara et al., *Proc. Nat. Acad. Sci. USA* 86:5673, 1989; Foh et al., *Science* 243:217, 1989) using 5' 3' RACE kits (Clontech, Palo Alto, CA; Life Technologies, Inc., Gaithersburg, MD) according to the supplier's instructions, with the following primers:

DSP12-GSP1:

5' GGC TGC TGG AGG C TGC TGC TGC TGC 3' SEQ ID NO:9

DSP12-SP4:

5' CCC AAC GCG GGG C TT TAT GAG GC AGC 3' SEQ ID NO:10

RACE products were amplified from the cDNA using these primers, indicating that sequences complementary to the portion of HIGS AC008081 identified in the tblastn search were present in the cDNA. Sequence information upstream and downstream from the conserved domain of HIGS AC008081 was also amplified in

DSP12-GSP2:

5' - GCCACAGCTTGTGTGGCCGCTGTGTTC - 3' SEQ ID NO:11

DSP12-GSP3:

5' - GCATTCATTCAGAGCCGAGATAAAGATGATCGAA - 3' SEQ ID NO:12

DSP12-GSP4:

5' - GGAGCTTGGCTTGTATGAGGCGCTCGG - 3' SEQ ID NO:13

10

DSP12revGSP1:

5'-GCA GAC AGA CAG CAG CCT CCA GCA GCC-3' SEQ ID NO:14

DSP12-SP6:

15 5' - GGTGGAGGAGACGGAAAGGGAGGAGGGC - 3' SEQ ID NO:15

DSP12-SP7:

5' - GGGAGCAGGGAGGTGGGGGCAGCTT - 3' SEQ ID NO:16

20

Sequences of the resulting RACE products indicated the presence in the cDNA of an open reading frame (Figure 1, SEQ ID NO:1) encoding a DSP-12 polypeptide of 552 amino acids (Figure 2, SEQ ID NO:2).

25

Repeating the HIGS database search with the DSP-12 catalytic domain sequence, as described above using the tblastn algorithm, indicated that portions of the genomic DNA sequence database entry having accession number HIGS AC007919 could also encode portions of the DSP-12 polypeptide sequence. Additionally, searching the HIGS database with the DSP-12 active site domain amino acid sequence as described above identified potential DSP-12 partial coding sequences in a variety

cDNA (SEQ ID NO:1) and HIGS AC010189, however, revealed an unexpected degree of sequence divergence on either side of the regions of high homology (Fig. 7). Sequence analysis of HIGS AC010189 was therefore performed to determine potential DSP coding regions of AC010189, and the following primers were prepared for 5' 3' RACE screening of human cDNA (reverse transcribed from human skeletal muscle) as described above:

DSP13-GSP1:

5' CTCTCCAGTTGCTCATGAAGCTTGGGTGGG 3'

10

SEQ ID NO:17

DSP13-GSP2:

5' GGGCTTGGTTACCGTTCGTCTTCTTTCACATAGTCA 3'

SEQ ID NO:18

15 DSP13-GSP3:

5'--GGATCACAATCATTTGCTATAAAATTCCTTGAATTCCTGC-3'

SEQ ID NO:19

DSP13-GSP4:

20

5' TGAATTCCTGCAAGTTCACACCATTTG 3' SEQ ID NO:20

DSP13-SP4:

5' GGAAATA TGGCTGGGAA TCTGGACCGAGCCATA TGA 3'

SEQ ID NO:21

25

DSP13-SP5:

5' TGTGAAAGAAAGACGAAACGGTAACCAAGCCAAAC 3'

SEQ ID NO:22

Sequences of the resulting RACE products indicated the presence of an open reading frame (Figure 3, SEQ ID NO:5) encoding a distinct polypeptide referred to herein as DSP-13. The DSP-13 polypeptide is homologous to, but distinct from, DSP-12. A DSP-13 polypeptide of 509 amino acids is shown in Figure 4 (SEQ ID NO:6). Additional RACE products that were obtained and sequenced revealed the presence of a DSP-13 alternate splice variant, the DNA sequence (Fig. 5A, SEQ ID NO:7) and 241 amino acid polypeptide sequence (Fig. 5B, SEQ ID NO:8) of which are shown in Figure 5.

The sequences of DSP-12 and DSP-13 had significant homology to other MAP-kinase phosphatases (Figure 6). DSP-12 and DSP-13 share a common active site domain (Fig. 7, positions 395-415) having the amino acid sequence:

CTVHC KMGVSR SASTVIAYAM

SEQ ID NO:3

Semiquantitative RT-PCR analyses were performed. These analyses showed detectable DSP-12 and DSP-13 encoding mRNAs in RNA samples from all human tissues analyzed, including brain, thymus, placenta, skeletal muscle, heart, liver, pancreas, testes and adipose tissue.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the present invention is not limited except as by the appended claims.